

Original Article

The Comparative Study of Binding Characteristics of Cobalt and Iron to Human Serum Transferrin

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ABSTRACT

Background: Cobalt (Co) is an essential trace element. Available data suggest an interaction between cobalt and other elements. In this study the characteristics of Co and iron (Fe) bindings to human apo-transferrin (apo-tf) have been investigated and compared.

Methods: Using spectrophotometric titration and equilibrium dialysis techniques, the bindings of Co(III) and Fe(III) to apo-tf were studied.

Results: Both Fe (III) and Co (III) as complexes with citric acid (1:20) were taken up by apo-tf, forming complexes with the maximum absorbances at 465 nm and 360 nm respectively.

Conclusion: The binding of iron to apo-tf at 465 nm was reduced by 20% when 225 nmole/ml of cobalt was added to the reaction mixture. The binding constant of iron to apo-tf was calculated using scatchard plot analysis and it was $22 \times 10^9 \text{ M}^{-1}$. The addition of cobalt (III), 200 $\mu\text{g/l}$ to the outside of the dialysis sac reduced iron Uptake by 30%. The binding constant of Co (III) to apo-tf was also calculated and it was $3 \times 10^9 \text{ M}^{-1}$.

Key words: cobalt-transferrin, iron-transferrin, binding activity.

Trace elements are required for life, and their existence seem to be essential for the development and growth of animals and humans. Cobalt is an essential trace element¹. This element occurs in the structure of cobalamine which is required for the activation of methylmalonyl-CoA mutase².

Available data suggest an interaction between cobalt and other elements. It has been previously reported that cobalt may interfere with iron^{3,4}, chromium, and manganese⁵. Cobalt may interfere with the metabolism of these elements either in intestinal absorption, during blood transportation, or other biochemical pathways.

The exact mechanism by which cobalt is transported across the blood circulation is still a matter

of speculation. It has been reported that albumin, α_2 -macroglobulin⁶, and transferrin are the major serum protein carriers for cobalt in the blood. The binding characteristics of cobalt to human transferrin have not been investigated comprehensively. Serum transferrin is a mammalian iron transport protein. It is a β_1 -glycoprotein with a molecular weight of approximately 80 KD⁷. It has two specific metal-binding sites that bind a variety of metal ions in addition to ferric ion⁸. These included aluminum⁹, chromium¹⁰, copper¹¹, gallium¹², manganese¹³, zinc¹⁴, etc. With regard to the above, the present project was undertaken to compare the binding characteristics of cobalt and iron to serum transferrin, using spectrophotometric titration and equilibrium dialysis techniques.

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Materials and Methods

All chemicals were purchased from Sigma Chemical Company, Germany.

Deionized water was used throughout the study for washing and preparing the solutions and also to minimize metal contaminations.

All glassware was soaked overnight in 3M nitric acid, and then thoroughly rinsed with distilled and deionized water. Plastic ware was pre-washed with 10mM EDTA, followed by three washing cycles with distilled and deionized water.

Preparation of APO-Transferrin

Human apo-transferrin was provided and further purified as follows. Human apo-transferrin (2.5 mg/ml) was dissolved in TRIS-HCl-bicarbonate medium (100 mM, pH 7.4), placed in prewashed vialing sacs, and dialyzed twice against 100 vol of 50 mM acetate buffer, pH 5.2, for 6 and 16 hours respectively. Protein solutions were then successively dialyzed against 100 vol of 0.15 mM NaCl, 0.02 M NaHCO₃ in 0.15 M NaCl, and finally TRIS-HCL - bicarbonate, (100 mM, pH 7.4)¹⁵.

The homogeneity of prepared apo-transferrin was checked by SDS-polyacryl-amide gel electrophoresis⁹.

Preparation of iron (III), and cobalt (III) citrate complexes

Separate stock standard solutions of Fe (NO₃)₃, 9H₂O (3mM), CoCl₂, 6H₂O (3mM) were prepared in deionized water containing 0.1 M HNO₃ and mixed with the equal volume of 60 mM citric acid solution. The solution were adjusted to pH 7.4 with 1M NaOH and made up to a final concentration of 1.5 mmole/L of iron and /or cobalt (II).

A cobalt (III) citrate stock standard solution was prepared by adding 4 ml of cobalt (II) citrate stock standard solution to 600 µl citrate buffer (1.2 mol/l, pH 5.8-5.9), followed by addition of 100 µl (2mol/1) of H₂O₂. This mixture was then made up to 5 ml with de-ionized water¹⁶.

Spectrophotometric titration technique of metals binding to APO-transferrin

The binding of metal ions to apo-transferrin was carried out using a Perkin-Elmer UV/VIS spectrophotometer (model 5515). The experiments were carried out at room temperature (22 ± 1^{0C}). Approximately 1.0 ml of freshly prepared apo-transferrin (2.5 mg/ml) in TRIS-HCl-bicarbonate (100 mM, pH 7.4) medium was added

to standard 1-cm prewashed acid glass cuvettes. To the cuvetts, aliquots (10-100 µl) of 1.5 mM metal ion as the citrate complex (1:20) were added and the volumes made up to 1.5 ml with the same buffer. The mixtures were mixed vigorously by vortexing. The cuvettes were capped and left for up to 1h at room temperature. The absorbances of the solutions were then determined at an appropriate wavelength.

Equilibrium dialysis technique

The binding of cobalt and iron to human serum apo-transferrin was also investigated using equilibrium dialysis at room temperature (22 ± 1^{0C}) in a chamber gassed with a 95% O₂ and 5% CO₂ mixture. A solution of apo-transferrin (2.5 mg/ml) was placed in a dialysis sac open to the atmosphere, which was immersed in a plastic vessel containing 1500 ml of TRIS-HCl-bicarbonate medium, pH 7.4. 300 µl of iron (1.5 mM) as ferric-citrate or cobalt (1.5 mM) as cobalt-citrate solutions were added at time intervals to the 1500 ml buffer surrounding the dialysis sac with the aid of a magnetic stirrer and was kept at the required pH by bubbling through the O₂ and CO₂ mixture. After 24h, 100 µl of sample was taken from inside and 100 µl of sample from outside the dialysis sac and analyzed for cobalt or iron concentrations. Iron was determined using phenanthroline as chromogen¹⁷. Cobalt level was determined by flameless atomic absorption spectrophotometry using perkin-Elmer model 3030. The binding constants of metals to apo-tf were calculated using Scatchard plot analysis¹⁸.

Results

The absorption spectrum of Fe (III) - transferrin complex was measured first. A broad peak at 465 nm was seen for Fe-transferrin complex in comparison to apo-transferrin. In the next series of experiment, the binding of iron to apo-transferrin in the absence and/or presence of cobalt (0-225 nmole/ml) as cobalt (III)-citrate were studied (Fig 1). It was found that the increase in the absorbance at 465 nm was reduced by approximately 20% when 225 nmole/ml of cobalt as cobalt-complex was added to the reaction mixtures (Fig 1).

The absorption spectrum of cobalt (III)-transferrin complex measured next.

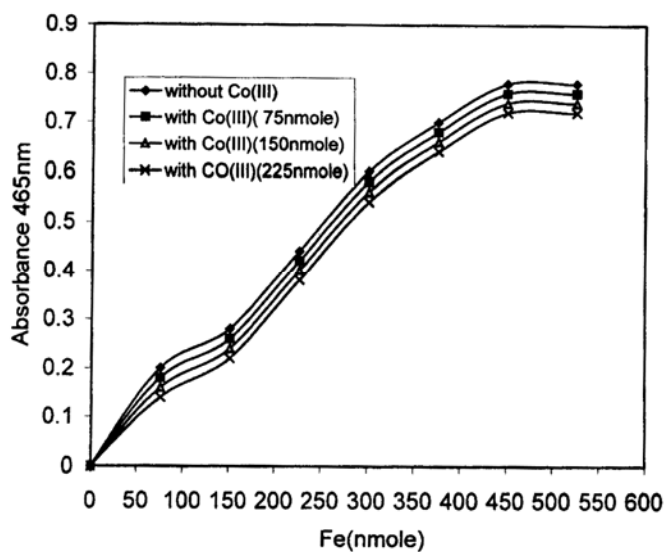


Figure 1. Spectrophotometric titration of human apo-transferrin with iron in TRIS-HCl-bicarbonate medium at pH 7.4 and effect of Co (III).

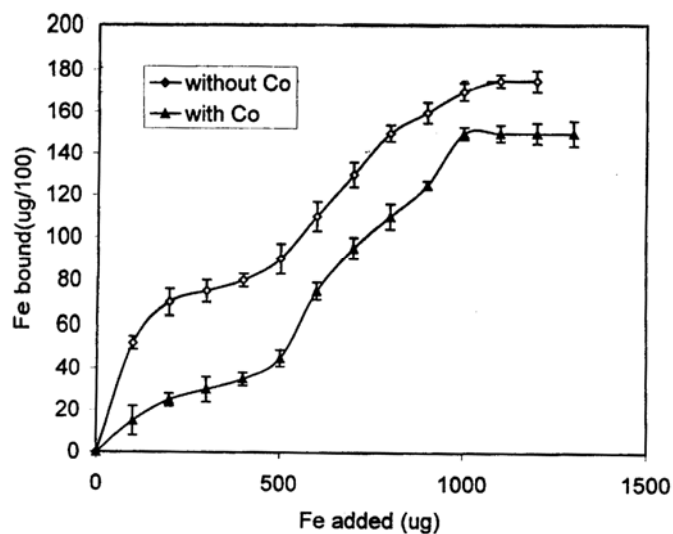


Figure 2. Equilibrium dialysis study. Binding of iron to human apo-transferrin in TRIS-HCl bicarbonate medium at pH 7.4; The effect of Co (III). Each point is the mean \pm SD of three experiments.

In comparison to apo-transferrin, a narrow peak at 360 nm was seen for cobalt (III)-citrate complex. In order to confirm further binding of iron (III), and/or cobalt (III) to apo-transferrin, equilibrium dialysis technique was set up next. The results obtained are presented in (Figures 2 and 3). Figure 2

shows the binding of iron to apo-transferrin and the effect of 200 μ g/l of cobalt (III)-citrate on the binding activity. Approximately a 30 percent reduction was seen in iron binding to apo-transferrin. Figure 3 shows the binding of cobalt to apo-transferrin and the effect of iron (200 μ g/l) on the cobalt (III) binding to apo-transferrin. Approximately a 20 percent reduction was seen in cobalt (III) uptake by apo-transferrin.

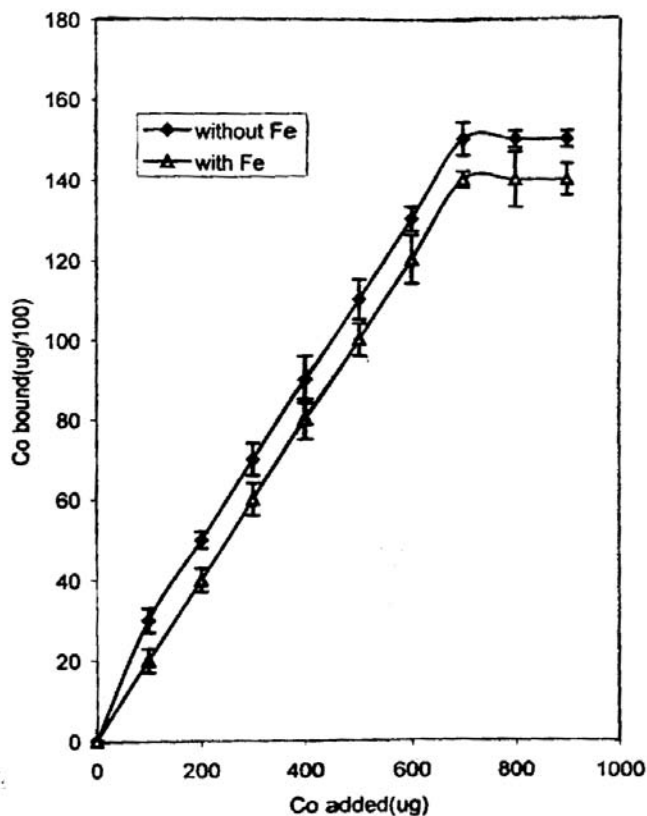


Figure 3. Equilibrium dialysis study. Binding of Co (III) to human apo-transferrin in TRIS-HCl bicarbonate medium at pH 7.4; The effect of Fe(III). Each point is the mean \pm SD of three experiments.

Using scatchard plot analysis and the data of figures 2 and 3, the binding constants for metal binding to apo-transferrin were calculated. To do this, free and bound metal ions to serum apo-transferrin were calculated and plotted as the ratio of Bound to free (B/F) versus bound cobalt ion to transferrin. The approximate binding constants obtained for iron and/or cobalt (III) to apo-transferrin complex were $22 \times 10^9 \text{ M}^{-1}$ and $3 \times 10^9 \text{ M}^{-1}$ respectively (Figures 4 and 5).

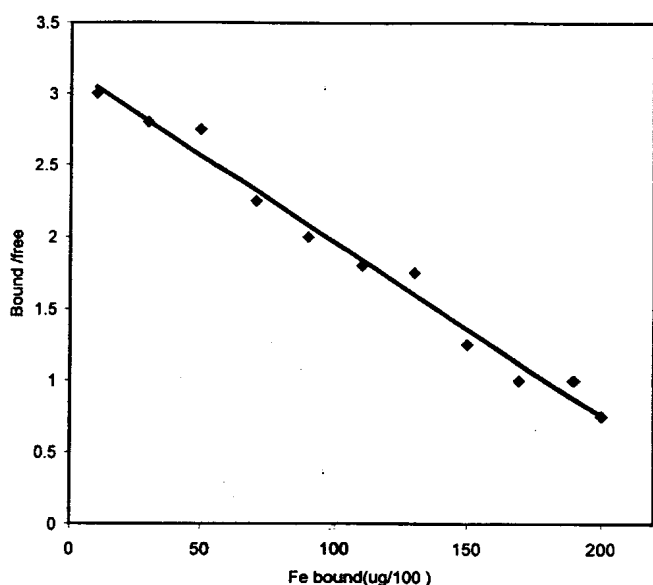


Figure 4. A typical scatchard plot for the iron binding to apo-tf.

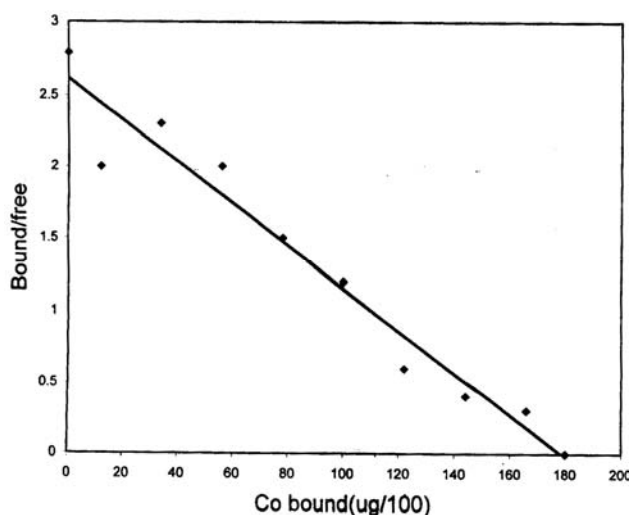


Figure 5. Scatchard plot for the Co (III) binding to apo-tf.

Discussion

Cobalt is an essential element. The absorption of cobalt is approximately 25% depending on the chemical form and content of the specimen. Toxicity of cobalt is relatively low. Cobalt, as an additive in beer, may cause toxicity in chronic beer drinking and also inhaling of large doses in work place exposure and may cause allergies, nausea, vomiting, renal failure, and hematological disorders, particularly anemia¹⁹. The exact mechanism by which the anemia occurs in those with cobalt overload is still a matter of speculation. The data that have been presented in this article elucidate the probable mechanism by which cobalt may interfere with iron metabolism. It has been found that cobalt binds to transferrin, which is a physiological iron supplier to red blood cells and also to hepatocytes since both tissues contains transferrin receptors²⁰. We found that the increase in the absorbance at 465 nm was reduced by approximately 20% when cobalt was added to the iron-transferrin complex, suggesting that cobalt may compete with iron to bind to transferrin molecules. The close relationship between cobalt-transferrin and iron-transferrin binding constants may also confirm the above suggestion. Previous reports from other laboratories also confirm the binding of cobalt to transferrin molecules using other biochemical techniques²¹. The appearance of anemia, however, may be due to either the interference with iron binding to transferrin, at the cell surface where by iron-transferrin binds to transferrin receptors²² or inside the cell where heme synthesis occurs. We are doing more investigations to elucidate the probable mechanism by which anemia occurs in cobalt overload people.

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